<Division of Environmental Chemistry>Molecular Microbial Science

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– Molecular Microbial Science –

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Scope of Research

Microorganisms are found almost everywhere on Earth. They have great diversity of capacities to adapt to various environments, including chemically and physically unusual environments. Our main subject is to clarify the molecular basis of environmental adaptations of microorganisms and their application. Specific functions of proteins and lipids with essential roles in environmental adaptation of extremophilic microorganisms are of our particular interest. We also undertake mechanistic analysis of microbial enzymes, in particular those involved in unique metabolic pathways, and their application.

KEYWORDS

Extremophiles
Bacterial Cold-adaptation Mechanism
Polyunsaturated Fatty Acid
Phospholipid Acyltransferase
Membrane Vesicle

Selected Publications

Ito, T.; Gong, C.; Kawamoto, J.; Kurihara, T., Development of a Versatile Method for Targeted Gene Deletion and Insertion by Using the *pyrF* Gene in the Psychrotrophic Bacterium, *Shwanella livingstonensis* Ac10, *J. Biosci. Bioeng.*, **122,** 645-651 (2016).

Ohke, Y.; Maruyama, S.; Tarui, J.; Wang, Y.; Kawamoto, J.; Kurihara, T., A Phosphoporin Homolog Plays a Critical Role in the Dissimilatory Iron-Respiration Linked to Iron (III) Reduction by a Cold-adapted Bacterium, *Shewanella livingstonensis* Ac10, *Trace Nutr. Res.*, **33,** 35-42 (2016).

Sugiura, M.; Park, J.; Kawamoto, J.; Esaki, N.; Kurihara, T., Regulatory Mechanism of Membrane Protein Production in an EPA-Producing Bacterium, *Shewanella livingstonensis* Ac10, *Trace Nutr. Res.*, **33,** 63-72 (2016).

Purification and Enzymatic Characterization of Bacterial 1-Acyl-*sn*-glycerol-3-phosphate Acyltransferase PlsC

A cold-adapted bacterium *Shewanella livingstonensis* Ac10 produces phospholipids esterified at the *sn*-2 position with eicosapentaenoic acid (EPA), which are important biomembrane components to survive in a cold environment. It is known that, in a bacterial *de novo* phospholipid synthesis, acylation at the *sn*-2 position is catalyzed by 1-acyl-*sn*-glycerol-3-phosphate acyltransferase PlsC. We previously carried out gene deletion experiments to find that the microbe has five PlsC homologs (PlsC1-5), among which PlsC1 is exclusively responsible for the production of the EPA-containing phospholipids. To gain further insights into the enzymatic properties of PlsC1, we attempted to purify and characterize the enzyme. After careful investigation of purification conditions, we finally succeeded in purifying PlsC1 in an active form and revealed its enzymatic features such as optimal reaction conditions and substrate specificity. It is notable that PlsC1 showed a higher activity toward unsaturated fatty acids including EPA than saturated ones. These findings will enable us to investigate a protein interaction network underlying the cold adaptation of *S. livingstonensis* Ac10 and to analyze as yet unidentified reaction mechanism of PlsC.

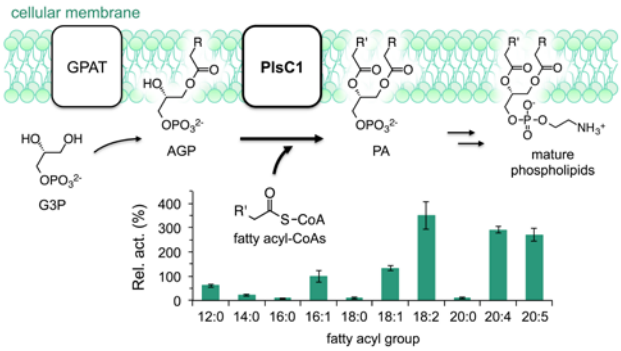

Figure 1. Schematic view of PlsC1-catalyzed reaction. PlsC1 showed a higher activity toward unsaturated fatty acids including EPA (20:5) and hexadecenoic acid (16:1) than saturated ones such as hexadecanoic acid (16:0). G3P, *sn*-glycerol 3-phosphate; AGP, 1-acyl-*sn*-glycerol 3-phosphate; PA, phosphatidic acid; GPAT, G3P acyltransferase.

Characterization of Membrane Vesicles Produced by *Shewanella* Species

It is known that most bacteria secrete membrane vesicles (MVs), which are composed of lipid membrane and contain nucleic acids, proteins and periplasmic solutes, to outer milieu. Though much attention has been paid to their physiological roles as well as the application to biotechnology, the molecular mechanism for MV secretion remains largely unknown. To better understand the mechanism, we characterized MVs from two cold-adapted *Shewanella* species. By using cryo-electron microscopy techniques, an EPA-producing bacterium *S. livingstonensis* Ac10 was shown to secrete MVs that mainly have a spherical single-bilayered structure with diameter of approximately 100 nm. Fatty acid profiling showed that specific fatty acids, e.g. hexadecanoic acid, are predominantly loaded onto MVs. Moreover, it was notable that the deletion of EPA biosynthetic genes altered the size and productivity of MVs. On the other hand, we found that *Shewanella* sp. HM13 secretes a much larger amount of MVs than *S. livingstonensis* Ac10. The MVs from this novel strain were found to include a single major protein. These features will be suitable for developing a new low-temperature protein production system where a protein of interest is concentrated in secreted MVs.

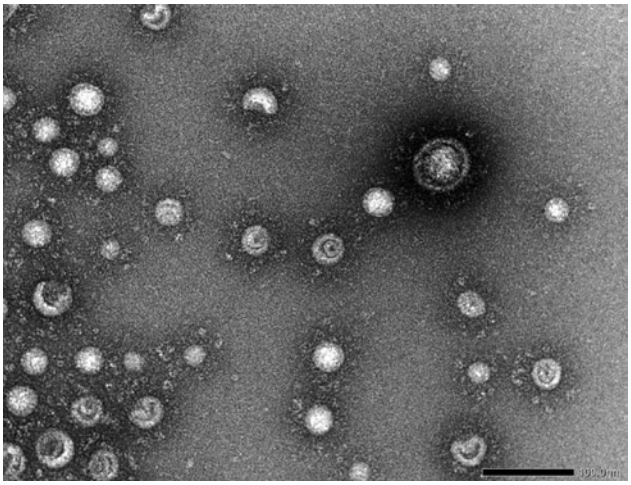

Figure 2. Electron microscopic image of MVs from *Shewanella* sp. HM13. The bar indicates 100 nm.